ECHA
EUROPEAN CHEMICALS AGENCY

Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level on toxicity to reproduction of

Epoxiconazole

EC number: 406-850-2
CAS number: 133855-98-8

ECHA/RAC/A77-O-0000001412-86-08/F

Adopted

28 November 2012

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OPINION OF THE COMMITTEE FOR RISK ASSESSMENT PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL ON TOXICITY TO REPRODUCTION OF EPOXICONAZOLE

Pursuant to Article 77(3)(c) of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (the REACH Regulation), the Committee for Risk Assessment (RAC) has adopted an opinion on toxicity to reproduction of epoxiconazole, confirming its previous opinions of 17 March 2010 and 11 March 2011[1].

I. PROCESS FOR ADOPTION OF THE OPINION

Following a request from the European Commission, in the mandate of 25 April 2012 attached as Annex 3, the Executive Director of ECHA asked RAC to develop and adopt an opinion on the classification and labelling of epoxiconazole, taking into account the previous RAC opinions, the additional information related to some new studies that has recently become available and the comments received during the public consultation.

The additional information related to some new studies was provided by the company BASF SE in the format of an Additional Information Report (AIR).

The AIR was made publicly available at: http://echa.europa.eu/harmonised-classification-and-labelling/consultation-following-echa-executive-director-request on **21 June 2012**. Parties concerned and MSCAs were invited to submit comments and contributions by **23 July 2012**.

II. ADOPTION OF THE OPINION OF RAC

Rapporteur, appointed by RAC: **Annick Pichard**

Co-rapporteur, appointed by RAC: **Christine Bjorge**

The RAC opinion takes into account the information on toxicity to reproduction provided by industry in the Additional Information Report (AIR) and the comments provided in the public consultation on the AIR and re-confirms its previous opinions that epoxiconazole fulfils the criteria for classification as toxic to reproduction 1B; H360D (CLP).

The RAC opinion was adopted on **28 November 2012**. It complements the RAC opinion of 17 March 2010 on a dossier proposing harmonised classification and

[1] RAC opinion No. CLH-O-0000000630-85-05/F of 17 March 2010

RAC opinion No. ECHA/RAC/A77-O-0000001412-86-02/F of 11 March 2011

labelling of epoxiconazole and the RAC opinion of 11 March 2011 on certain scientific study plans in relation to epoxiconazole.

The RAC opinion was adopted by consensus.

III. OPINION OF RAC

After examination of the information on toxicity to reproduction made available in the additional information report (AIR) and the comments received in the public consultation, the Committee confirms that its conclusion regarding the classification and labelling of epoxiconazole for toxicity to reproduction in its opinions of 17 March 2010 and of 11 March 2011 was based upon a proper evaluation of the data and that additional information recently made available has convinced the RAC to confirm its previous conclusions.

History of the opinion

Based on the dossier submitted by Sweden, in March 2010 the RAC adopted by consensus the opinion on harmonised classification and labelling of epoxiconazole as toxic to reproduction in category 1B for developmental toxicity.

In March 2011, based on the mandate from the Executive Director of ECHA, RAC adopted an opinion on certain scientific study plans in relation to epoxiconazole and concluded that it seemed unlikely that the harmonised classification and labelling already adopted would be modified as a result of the studies.

In March 2012 RAC was again requested, pursuant to Art. 77(3)(c) "*to develop and adopt an opinion on the classification and labelling of epoxiconazole, taking into account the previous RAC opinions, the aforementioned additional information [AIR, related to a number of new studies] that had recently become available and the comments received during the public consultation*".

Justification

Two main adverse effects of epoxiconazole on development are considered as critical for the classification on developmental toxicity: 1) post implantation losses and resorptions and 2) malformations (cleft palates); while three main issues related to these effects are dealt with in both cases: a) maternal toxicity, b) choice of the guinea pig as a test species and c) mode of action for the two types of effects.

<u>Post implantation losses and late resorptions</u>

The late resorptions/post-implantation losses as demonstrated in the rat were reduced or diminished when rats were co-administered with oestradiol and were not seen in guinea pigs. Data on post implantation losses had also been shown in a non-human primate (baboon) with another aromatase inhibitor, letrozole. RAC considered that the guinea pig may not necessarily be a better model in relation to humans than the rat for this effect. It was recognised that rats, guinea pigs, non-human primates and humans are sensitive to the general mode of action of aromatase inhibition and that physiological species differences may lead to various responses although through a common mechanism.

<u>Cleft palates</u>

It was re-confirmed by RAC, that the high incidence of cleft palates in rats was seen as sufficient evidence for classification as Repr. 1B as was already concluded under the first (2010) Art. 77(3)(c) mandate on epoxiconazole. This malformation is rarely seen in rats. This result has been confirmed in one of the new rat studies (and shown to be independent of co-administration of oestradiol). Skeletal findings had been shown in the guinea pig. No clear mechanism for the cleft

palates had been demonstrated and the possible relevance of this malformation (as also observed with other substances of the azole class) to humans cannot be excluded.

Comparison with the CLP criteria

The two main adverse effects 1) **post implantation losses and late resorptions** and 2) **cleft palates** have been assessed and compared with the CLP criteria by the Committee and the conclusions are as follows:

- For post implantation losses and late resorptions, taking into account the similar effect seen in rats and for another azole (letrozole) in non-human primates, the common mode of action to all test species, as well as the relevance to humans, a classification as **Repr. 1B (CLP)** is proposed for this effect.
- The presence of cleft palates in the rat foetuses in the presence or absence of overt maternal toxicity, the presence of skeletal findings in guinea pigs (e.g. fusion of thoracic centrum and arch) in the absence of a clear mechanism of action explaining the induction of anomalies, all support classification as **Repr. 1B (CLP).**
- In conclusion, it is proposed to classify **epoxiconazole** as **Repr. 1B (CLP).**

Conclusion

The Committee re-confirms the classification and labelling of epoxiconazole (EC number: 406-850-2, CAS number: 133855-98-8) as toxic to reproduction 1B; H360Df according to the CLP Regulation and toxic to reproduction Cat. 2, R61 (Directive 67/548/EEC) as recommended in the previous opinions of 17 March 2010 and 11 March 2011.

IV. SCIENTIFIC GROUNDS FOR THE OPINION

Conclusions of the RAC opinion of 17 March 2010

With regard to reproductive toxicity, RAC considered in its opinion of 17 March 2010 that epoxiconazole should be classified in Category 1B (CLP criteria) (and in Category 2 according to Directive 67/548/EEC) with regard to developmental toxicity, in addition to the existing EU harmonised classification for fertility in Category 2 (CLP criteria) (and Category 3 according to DSD criteria) in Annex VI, CLP.

Two main adverse effects of epoxiconazole on development were identified and considered as critical for the agreed classification on developmental toxicity:

- Post implantation losses and resorptions
- Malformations in particular cleft palates

In several rat studies a clear post-implantation loss was observed consisting of late or very late resorptions. In some of these studies, the effects occurred in the absence of significant maternal toxicity. Epoxiconazole is an aromatase inhibitor and RAC noted that an increase in late resorptions was also induced by another aromatase inhibitor. Thus, the late resorptions in rats may be linked to an endocrine disruptive effect of aromatase inhibitors by decreasing oestradiol levels in the dams. Because of species differences in hormonal regulation of gestation, it could be argued according to RAC that a doubt on human relevance could be raised for such a mechanism of action. However in the absence of clear data to establish the mechanism of action of epoxiconazole for induction of late resorptions, RAC could not make any conclusion on the potential absence of relevance for humans.

A very high rate of cleft palates (50% of foetuses, in 90% of litters) was observed in a rat study at 180 mg/kg/day which also caused maternal toxicity (a significant decrease in the corrected body weight gain). Isolated findings of cleft palate without maternal toxicity were observed at a lower dose in the same study and at lower doses also in other studies on epoxiconazole. RAC noted that cleft palate is a rare malformation in rats, which however is commonly observed in studies with triazole compounds in the presence or absence of maternal toxicity. RAC concluded that cleft palate could be clearly identified as a developmental effect of epoxiconazole, which could not be considered secondary to maternal toxicity manifested as decreased food consumption, reduced body weight gain or other toxic effects. In the absence of relevant mechanistic information RAC could not conclude on the potential absence of relevance for humans.

RAC considered that the level of evidence for induction of both post-implantation losses and cleft palates by epoxiconazole is in agreement with the criteria for classification as Repr. 1B (CLP) for developmental toxicity.

Conclusions of the RAC opinion of 11 March 2011

Upon a request (according to Article 77(3)(c) of REACH) RAC reviewed six study plans in rats and guinea pigs, aiming to clarify the mechanism of action of post-implantation losses and cleft palates observed in rats and whether the mechanisms were species specific.

RAC considered that the additional studies described in these study plans were relevant with respect to one of the questions raised by RAC concerning late resorptions, whilst the potential for a teratogenic effect (cleft palate) of epoxiconazole may remain unexplained.

Concerning the study plans on guinea pigs, RAC noted that lack of relevance for humans would not be shown as such if the guinea pig studies should not show embryo/foetal mortality, because there was no clear rationale presented in order to assume that guinea pigs are more similar to humans than rats. Depending on what mechanism(s) of action that is (are) identified, the data may, or may not decrease the concerns for human relevance for the late resorptions.

The suggested mechanisms potentially implied for the malformations caused by azole derivates at high dose levels in experimental animals and in humans (e.g. by fluconazole) may be different from or complementary to those inducing the late resorptions. However, this would not be explored in either test species.

RAC concluded that the previously agreed proposal for a harmonised classification of epoxiconazole in Repr. 1B (CLP) seems unlikely to be modified by the result of the six studies.

Summary of the Additional Information Report (AIR) submitted by BASF SE

BASF SE has generated new toxicological data in the process of clarifying the endocrine disruption potential of epoxiconazole according to the requirements of Annex I of Commission Directive 2008/107/EC[2]. BASF has been requested to submit all data available by 1 March 2012 to the EU for the purpose of data evaluation and consideration of the appropriate classification and labelling of epoxiconazole by the Committee for Risk Assessment (RAC).

[2] Commission Directive 2008/107/EC of 25 November 2008 amending Council Directive 91/414/EEC to include abamectin, epoxiconazole, fenpropimorph, fenpyroximate and tralkoxydim as active substances

The new study data was considered to provide useful and relevant information. Robust summaries of these investigations are included in the AIR (Annex 6).

In Annex 4 of this opinion the Committees' comments on the data provided by the submitter of the AIR are summarised.

The data from the submitter of the AIR related to the two main adverse effects are discussed and commented in detail further below under the following headings.

Comments received during the public consultation on the Additional Information Report (AIR)

During the public consultation on the Additional Information Report (AIR) five comments were received:

- Two Member States (MS) supported classification Repr. 1B; H360;
- One MS could support the proposed classification on the already adopted RAC opinion based on the data on cleft palates;
- Two comments from industry did not support classification Repr. 1B.

The RAC responses to the comments are included in the RCOM document (Annex 5).

Outcome of this RAC assessment

The two key adverse effects 1) post implantation losses and resorptions and 2) malformations, in particular cleft palates are discussed in the opinion while the following three aspects related to these effects were taken into consideration:

a) The choice of guinea pigs as a model for toxicity to reproduction model for the study of developmental toxicity of epoxiconazole

b) The mode of action of epoxiconazole and the relevance to humans

c) The definition of maternal toxicity and its role in the toxicity of epoxiconazole.

1. Impact of epoxiconazole on post implantation losses and resorptions

Previous studies on rats and summary of the first RAC opinion of 17 March 2010

Several prenatal developmental toxicity studies in experimental animals are available and provide information on the induction of post-implantation losses.

By the oral route, no significant increase in post-implantation losses was observed in studies where rats were exposed to 45 mg/kg/d of epoxiconazole (Hellwig 1990b) and to 180 mg/kg/d (Hellwig 1989) from gestation days (GD) 6 to 15. However, a large increase of post-implantation losses was observed in Schneider 2002 at the same dose of 180 mg/kg/d with an exposure partially extended to the end of gestation (GD 6-19). Resorptions were mainly identified as late resorptions.

In Taxvig 2007 and 2008, where exposure was entirely extended to the end of gestation (GD 7-21), a significant increase in post-implantation losses was observed at 50 mg/kg/d and consisted in late and very late resorptions.

No effect was observed in rats by the dermal route up to 1000 mg/kg/d (Hellwig 1993).

An increase in post-implantation losses was also observed at the highest dose by the oral route in rabbits in presence of maternal toxicity (Hellwig 1990a) and consisted mainly of early post-implantation losses in contrast to rats.

In the rat two-generation study (Hellwig 1992) a significant decrease in mean litter size was observed at the highest dose in F1a and F1b that may be consistent with an effect on post-implantation losses.

Altogether, these data indicate that the induction of post-implantation losses by epoxiconazole is worsened when the duration of exposure is extended to the end of gestation with higher rate of resorptions and later stages of resorptions observed. Post-implantation losses were observed in prenatal developmental toxicity studies, in which dams were sacrificed before parturition. It is considered that dystocia may not have contributed to the induction of resorptions. Induction of post-implantation losses was observed in the Taxvig studies in absence of significant maternal toxicity. Therefore, it cannot be considered secondary to nonspecific maternal toxic effects. In these studies, maternal toxicity was assessed by measurement of maternal body weight gain and clinical signs but it should be noted that maternal food consumption was not measured.

The hypothesis that this effect could be secondary to endocrine disruptive effects in the mother has been raised. However, no correlation between the progesterone level in dam plasma and the rate of very late resorptions was identified in an analysis of individual data from the Taxvig 2007 and Taxvig 2008 studies. It should however be noted that available data on hormonal effects of epoxiconazole in dams show a consistent significant effect on oestradiol and testosterone levels but not on progesterone. In Schneider 2002 both oestradiol reductions and induction of late resorptions were observed. Besides, another aromatase inhibitor – letrozole - has effects on maternal levels of oestradiol but not on progesterone in monkeys (Albrecht 2000). In rats, letrozole also induces an increase in late resorptions that was prevented by co-exposure to oestrogen (Tiboni 2009). This tends to demonstrate that late resorptions in rats may be linked to endocrine disruptive effect including an aromatase inhibiting action of epoxiconazole in the dams leading to a depletion of oestradiol. It can be argued that due to differences in hormonal regulation of gestation between species, a doubt on human relevance could be raised for such a mechanism of action. However, in absence of clear data to establish the mechanism of action of epoxiconazole for induction of late resorptions, the relevance to humans could not be ruled out.

Additional Information Report (AIR)

Considering the results of the Taxwig studies, two studies on rats were provided by the submitter of the AIR:

1) A study on pregnant rats (Schneider et al., 2010a and Schneider and Moreno 2011) at doses of 23 and 50 mg/kg/bw/d of epoxiconazole by oral route from GD 7 to GD 18 or 21 using the vehicles CMC (carboxymethyl cellulose) or corn oil.

At 50mg/kg bw/d, there was a clear increase in late resorption and a decrease in the percentage of live foetuses. When treatment covered only organogenesis (GD 7-18) with epoxiconazole dissolved in corn oil, there was a statistically significantly increased number of late resorptions (36.4% vs. 0% in control) and post implantation losses (39.7% vs. 9.8% in control) and a statistically significantly decreased percentage of live foetuses (about 30% less than control). When epoxiconazole was dissolved in CMC, there was a statistically significantly increased number of late resorptions (32.7% vs. 0% in control), an increased post implantation losses (34.8% vs. 10% in control) and a slightly decreased percentage of live foetuses (about 14% less than control).

When the treatment was extended to the end of pregnancy (GD 21) and using the corn oil as the vehicle, there was a statistically significantly increased incidence of late resorptions (35.8% vs. 2.5% in control) and post implantation losses (42.1% vs. 8.0% in control) and a statistically significantly decreased

number and percentage of live foetuses (7.4 vs. 11.5 foetuses in control, 31% less than control) at 50 mg/kg bw/day. When using CMC as the vehicle, there was a statistically significantly increased number of late resorptions (27.7% vs. 0.4% in control) and post implantation losses (32.3% vs. 6.2% in control) and a statistically significantly decreased percentage of live foetuses (22% less than control) at 50 mg/kg bw/day. Maternal toxicity was induced by epoxiconazole at both doses. Maternal toxicity was described as decreased food consumption, decrease in carcass weight and corrected net weight gain, and decreased platelet count. Eventually, in all groups treated with epoxiconazole and regardless of the vehicle used, there were necrobiotic placentae which could be associated with blood coagulum around placenta at necropsy.

Histopathological examination of placentae showed a dose dependent severity of the degeneration of the labyrinth and the trophospongium. The degenerative effects seemed also more pronounced in placentae with late resorption evident as severe to massive degeneration than with live foetuses evident as slight to moderate degeneration.

Decreased oestradiol and progesterone values, increased androstenedione and testosterone values were also observed in both formulations (corn oil or CMC). The severe decreases in oestradiol levels were dose dependent at GD 18 and close to 100% at GD 21 at both dose levels. The decrease in progesterone levels and the increase in androstenedione levels were independent of the duration of the treatment and without clear dose dependence. There was a trend to an increase in the testosterone level at GD 18, the effect at GD 21 being unclear.

It was concluded that the results of this study were in accordance with Taxwig conclusions in 2007.

2) A study (Schneider et al., 2010b and Schneider, Moreno and Fabian, 2011) on pregnant rats at a dose of 50 mg/kg/bw/d of epoxiconazole by oral route with co-administration of oestradiol cyclopentylpropionate (ECP) at doses of 0, 0,5 or 1 µg/animal/day by subcutaneous injection.

In this study maternal toxicity was demonstrated as a decrease in the oestradiol values and an increase in androstenedione and progesterone values. Clinical signs (vaginal hemorrhage and piloerection) and decreased food consumption were observed in all epoxiconazole treated groups, independent of the dose of ECP administered. Mean body weight and mean net body weight gain (about 58% below control values) were only affected in the epoxiconazole treated group without ECP supplementation. The effects on the haematology and clinical chemistry parameters were comparable in all epoxiconazole treated groups with a slightly more marked effect when ECP was not co-administered. Oestradiol levels were drastically reduced when compared to controls and dependent on the dose of ECP administered. The increase in the androstendione and progesterone levels was also ECP dependent.

Table 1, Hormones (GD 21; see table 2/22 in the AIR, page 48)

	Controls	Epoxiconazole 50 mg/kg/day	Epoxiconazole 50 mg/kg/day + 0.5µg ECP/animal/day	Epoxiconazole 50 mg/kg/day+ 1 µg ECP /animal/day
Esgtradiol (pmol/L)	41.0	0.0 (-100%)	0.2 (-100%)	2.2 (-95%)
Androstendione (nmol/L)	5.1	4.6	6.6 (+28%)	6.7 (+30%)

Progesterone (nmol/L)	132.2	161.6	242.8 (+84%)	252.0 (+91%)

In the group receiving 50 mg/kg of epoxiconazole without ECP supplementation, there was a statistically significantly increased number of late resorptions (2.9% or 30.1% vs. 0.1% or 0.6% in control) and post-implantation losses (51.6% vs. 6.3% in control). Consequently, a statistically significant decrease in the percentage of live foetuses (about 33% less than control) was also observed. There was no adverse consequence of the above-mentioned changes on live foetuses in any of the treatment groups receiving EPC alone (0.5 or 1 µg/animal/d) or epoxiconazole supplemented with EPC (0.5 or 1 µg/animal/d). The histopathological examination of dams and placentae revealed the following effects:

- Significant absolute and relative weight increases in the liver in the control group supplemented with 1 µg/animal/d ECP and in the liver and adrenal glands of all groups treated with epoxiconazole supplemented or not supplemented with ECP. All of these changes were considered to be treatment-related.
- Enlarged placentae and placental degeneration in all groups treated with epoxiconazole dependent on the dose of ECP co-administered. Placental degeneration consisted of a cystic dilation of maternal sinuses and rupture of interhemal membrane in the labyrinth with concomitant changes in the trophospongium. The same pattern of alteration was observed in placentas with live foetuses as in placentas with late resorptions, however the effects were considered more pronounced in the latter.

RAC comment:

There is a correlation between the oestradiol reduction and the foetal mortality. The supplementation of ECP during pregnancy prevents foetal mortality and the supplementation of ECP tends to reduce the severity of degenerative placental changes caused by epoxiconazole.

3) Three studies **on guinea pigs** were also provided by the submitter of the AIR: The first modified prenatal toxicity study was a range-finding study of epoxiconazole by oral route at doses of 5, 15, 50 and 180 mg/kg bw/d (Schneider et al., 2011a)

At 180 mg/kg bw/d one third of the animals died until mid-pregnancy and caused distinct signs of intoxication in the surviving animals in this dose group.

Based on these effects, doses of 15, 50 and 90 mg/kg bw/day were chosen for the definitive modified prenatal developmental toxicity study.

The only remarkable observations were alterations in steroid hormones starting at the dose of 5 mg/kg bw/d.

RAC comment:

It should be noted that the dose of 180 mg/kg/day which was also used in rat studies induced higher mortality in pregnant guinea pigs compared to pregnant rats, probably related to a higher internal exposure in guinea pigs. It should also be noted, that from the lowest dose level the hormonal balance (corticoids and steroids) was altered.

4) In the second modified prenatal toxicity study, pregnant **guinea pigs** were exposed by oral route to 15, 50 and 90 mg/kg bw/d of epoxiconazole (Schneider et al., 2011b).

The dose of 90 mg/kg bw/d caused a mild anemia and there were signs of a higher steroid hormone production of the adrenals.

The no observed adverse effect level (NOAEL) for maternal toxicity was set at 50 mg/kg bw/d.

Steroid hormone level alterations were observed starting at a dose of 15 mg/kg bw/d. The increase of oestradiol precursors such as testosterone and androstenedione may suggest that guinea pigs are sensitive to aromatase inhibition. It is noteworthy, that contrary to rats, histopathology of the placenta did not reveal any adverse effect caused by epoxiconazole up to and including a dose of 90 mg/kg bw/d.

RAC comment:

This study clearly shows that, similarly to rats, guinea pigs are sensitive to aromatase inhibition and that the steroid hormone cascade is altered as in other test species. The absence of placental effects as seen in rats is likely due to the species differences in morphology and physiology of this organ.

5) In the third study pre and post natal developmental toxicity pregnant **guinea pigs** were exposed by oral route at doses of 15, 50 and 90 mg/kg bw/day of epoxiconazole from GD 6 to end of gestation and continued through weaning (postnatal day 21) (Schneider et al., 2011c).

The no observed adverse effect level (NOAEL) for maternal toxicity was 15 mg/kg bw/d.

No effects were noted on gestation, parturition and up-bringing of offspring, at the tested dose levels up to 90 mg/kg bw/d.

RAC comment:

Again, as the above mentioned studies, this study also demonstrates that guinea pigs are sensitive to the aromatase inhibition and the perturbation of the steroids cascade induced by epoxiconazole.

General RAC comments on the choice of guinea pigs, the mode of action and maternal toxicity:

a) The choice of guinea pigs

Guinea pigs are used by the submitter of the AIR as an animal model for studying the developmental toxicity of epoxiconazole. The submitter of the AIR considered that the hormonal regulation of pregnancy and parturition in guinea pigs are more similar to humans than rats are to humans.

The choice of guinea pigs is the main argument for the submitter of the AIR to demonstrate that embryo foetal deaths seen in rats exposed to epoxiconazole are due to the depletive effect on oestradiol levels (through aromatase inhibition). These studies are considered by the submitter of the AIR to demonstrate that guinea pigs can be a good model for the evaluation of compounds which affect the maintenance of pregnancy in murid models, the placentation being more similar between guinea pigs and humans than between murids and humans.

RAC comment:

According to OECD Test guidelines, for regulatory purposes rats or rabbits are normally used as animal models for the evaluation of reproductive and developmental toxicity. The use of these animal models has many advantages including study design, easily available historical control data, short gestation, and large litter size. However, guinea pigs are shown to be sensitive to teratogenic effects induced by some chemicals and may be used as an

alternative animal model for the evaluation of reproductive and developmental toxicity (Kronick et al., 1987; Smith et al., 1992; Byrnes et al., 2001).

The guinea pig animal model is used in regulatory reproductive toxicity testing for studying the role of steroid hormones during pregnancy and parturition (Rocca and Wehner 2009). Guinea pigs have physiological characteristics of gestation that are considered similar to the human pregnancy. There are known differences in the hormonal regulation of late pregnancy between rats on one side and guinea pigs and humans on the other side. However, the experience with the use of guinea pigs as an animal model for reproductive and developmental toxicity is limited. The historical control data is very scarce due to the limited number of reproductive toxicity studies performed with guinea pigs. This contributes to a significant limitation of the evaluation of the study results with guinea pigs following exposure to epoxiconazole. Guinea pigs also show variability in pregnancy rates, small and variable litter size and have a long gestation.

b) The mode of action

RAC considered that the main point is that rat, guinea pig and non-human primate and human are sensitive to the general mode of action of aromatase inhibition:

- Hormonal changes (decreased oestradiol, increased testosterone) are observed in both the rat and the guinea pig studies with epoxiconazole. In addition, letrozole administered to baboons during pregnancy induces miscarriages in 62 % of the females during the first half of pregnancy and in 25 % in the second half (Albrecht, 2000). A co-treatment with estrogen fully abolished the adverse effect and all treated females gave birth to viable newborns. The larger impact of estrogen suppression on pregnancy maintenance in the first half of gestation demonstrated in this primate may reflect a gestational age-specific role for estrogen on uteroplacental blood flow, placental villous vascularization (Albrecht et al, 2004 and Robb et al, 2004) and /or extravillous trophoblast migration and invasion of uterine spiral arteries (Albrecht et al, 2006) in early pregnancy. This regulatory role may vary between species and placental type. Although there are no data with epoxiconazole in non-human primates, an embryotoxic effect of letrozole has been demonstrated in non-human primates and thus the likelihood of a similar effect on human pregnancy is high.
- Aromatase inhibitors are widely used for therapeutic uses in particular in the treatment of breast cancer for its ability to block estrogen production.

The consequences of such inhibition can differ from one species to another depending on the differences in the hormonal regulations of physiological process.

c) Maternal toxicity

The submitter of the AIR considered that the results of the prenatal development toxicity in rats established a correlation between maternal toxicity (in the presence of hormonal change) and the increase of late resorptions. This effect is not observed in guinea pigs due to the mechanism of hormonal regulation which is more relevant to humans.

Moreover, the new data on rats provide evidence that marked depletion of maternal oestradiol levels resulting from epoxiconazole–mediated aromatase inhibition is causally related to placental damage and to late foetal death in rats.

Based on the results, the submitter of the AIR concludes: in view of the known differences in the hormonal regulation of pregnancy between rats and humans,

the demonstrated mechanism of action for induction of late foetal resorptions in rats is considered to be of no or very limited relevance to humans.

RAC comment:

The contention by the submitter of the AIR that the toxic effects are observed together with maternal toxicity needs further consideration. These effects are limited to decreased food consumption, decrease in carcass weight, corrected net weight gain, and decreased platelet count. These effects are not sufficient to explain the occurrence of the post-implementation losses (Beyer et al, 2011). Moreover, the results of the rat studies are important since the post implantation losses and resorptions are not linked to the parturition process. In rats, letrozole also induces an increase in early and late resorptions that is prevented by co-exposure to estrogen (Tiboni 2008 and 2009). The baboon data for letrozole further indicates that the rat model is valid since the compound induces miscarriages in this species during early and late gestation (Aberdeen et al., 2010). Despite differences between species (e.g. hormonal balance, placentation), there are similarities in adverse effects on pregnancy between rodents and primates which, with the common mode of action (aromatase inhibition) seen in all test species, reinforce the relevance to humans.

In conclusion, taking into account the similar effect seen in rats and the common mode of action in all test species, as well as the relevance to humans, a classification **Repr. 1B (CLP)** is proposed for this endpoint.

2. Impact of epoxiconazole on malformations in particular cleft palates

Previous studies in rats and reminder of the first RAC opinion of March 2010

Several prenatal developmental toxicity studies are available and provide information on the induction of cleft palates.

A very high rate of cleft palates (50 % of foetuses, 90 % of affected litters) was observed in rats by the oral route in Hellwig (1989) at the high dose of 180 mg /kg bw/d. Such an increase was not reproduced at the same high dose in Schneider (2002). In this study two purity batches were used and in these two, cleft palates were only observed in 2 (2,4 %) and 1 (0,8 %) foetuses. However, in this study the high rate of post implantation losses (respectively 59% and 43 %) may have masked teratogenic effects. Maternal toxicity was noted at the high dose level in both studies demonstrated as a decrease in food consumption and significant decrease in corrected maternal body weight gain (- 45% and – 30%). One cleft palate was also observed in the low dose (20 mg/kg/d) in Hellwig (1989).

In other prenatal development toxicity studies, one cleft palate was also identified in the mid-dose (15 mg/kg/d) in rats by oral route in Hellwig (1990 b), and one in the high dose (1000 mg/kg/d) in rats by dermal route (Hellwig, 1993). Furthermore one cleft palate was reported in the two generation study (Hellwig, 1992) in the highest dose in F1b (approx. 23 mg/kg bw/d). No maternal toxicity was observed at these dose levels in these rat studies.

In rabbits, one cleft palate was observed at the low dose (5mg/kg bw/d) by the oral route (Hellwig, 1990 a). However, in the absence of such findings at the mid- and high dose its significance is unclear.

Cleft palate is a rare malformation with available historical control data in rats showing that 1 foetus with a cleft palate may be spontaneously observed on rare occasions (historical control mean: 0.06%; range: 0-0.2.% according to Hellwig (1990b) indicating 1 cleft palate observed in 2 out of 10 studies). The occurrence of one cleft palate in one study is therefore consistent with historical control

values and cannot be unequivocally attributed to the treatment. However, the repetition of this isolated finding in all five rat prenatal developmental toxicity studies that investigate malformations supports the conclusion that they are not of spontaneous origin and that they are biologically significant.

The absence of a dose-related response in two of the studies (Hellwig 1989 and Hellwig 1990b) also raises some uncertainty with regard to the relation of this malformation to the treatment. However, considering the generally low occurrence of this finding, a very large number of animals would be needed in order to observe a clear dose-related response.

In addition, cleft palate is a malformation that is commonly observed with triazole compounds in the presence or in the absence of maternal toxicity. It is a very specific malformation implying a disturbance in the process of craniofacial morphogenesis and several modes of action have been proposed. Menegola 2006 suggested that triazoles may inhibit the embryonic CYP450 (CYP26) involved in the regulation of retinoic acid whereas an alternative hypothesis involving blockade of IKr potassium channel, embryonic arrhythmia and hypoxia has also been proposed based on data for ketoconazole (Ridley 2006, Danielsson 2007).

However, none of these modes of action have been studied following exposure to epoxiconazole.

Overall, RAC considers that based on a weight of evidence approach and considering the specificity and the spontaneous infrequency of this malformation also seen with other triazoles, the induction of a high incidence of cleft palates in the presence of maternal toxicity (Hellwig 1989) and the repeated observation of isolated cleft palates in rats at doses without maternal toxicity enable **a clear identification of cleft palate as a developmental effect** of epoxiconazole. It is considered that induction of cleft palates cannot be attributed to maternal toxicity such as decreased food consumption or reduced body weight gain and **it cannot be considered secondary to other maternal toxic effects**.

Additional Information Report (AIR)

The submitter of the AIR provided several studies:

1) A prenatal developmental toxicity study in rats (Schneider et al., 2010c)

One group of pregnant Wistar rats were administered epoxiconazole by daily oral gavage at dose levels of 180 mg/kg from GD 6-15. Two other groups received epoxiconazole (180 mg/kg orally) plus daily subcutaneous injection of 1 or 2 µg/rat/day oestradiol cyclopentylpropionate (ECP).

The external malformations were markedly increased in all groups dosed with epoxiconazole (47-64% of the litters showed cleft palates, compared to 0% in controls); highest values (64%) were obtained in the group co-administrated the high oestradiol dose (2 µg/rat/day).

Signs of maternal toxicity were observed at 180 mg/kg bw/d and were manifested in the form of reduced feed consumption, and reduced corrected body weight gain (between 37-71%) which corresponds approximately to a 5% to 7% decrease in absolute mean body weight when compared to controls. Such decreases cannot be considered as severe maternal toxicity, as described by Beyer et al., 2011.

RAC comment:

In the RAC view, the new BASF study confirms that epoxiconazole is a potent teratogen in rats at 180 mg/kg. The observed signs of maternal toxicity (e.g. decreased body weight gain) cannot explain the very high incidences of cleft palate and other manifestations observed in rats at this dose level.

RAC is of the opinion that the results at 180 mg/kg clearly indicate that cleft palate is independent of hormones regulation (oestradiol).

The mechanisms for the induction of cleft palates and resorption is therefore clearly different.

2) A prenatal developmental toxicity study in guinea pigs (Schneider et al., 2011b)

Epoxiconazole was tested for its prenatal developmental toxicity in Dunkin Hartley guinea pigs. The test substance was administered in 1% carboxymethylcellulose suspension in highly deionized water (1% CMC) at doses of 15, 50 and 90 mg/kg body weight on gestation day GD 6 through GD 63.

The dose of 90 mg/kg bw/d caused a mild anemia and signs for a higher steroid hormone production of the adrenals, possibly related to stress in pregnant guinea pigs. Unlike in rats, histopathology of all placentas did not reveal any adverse effect caused by epoxiconazole up to and including the highest dose of 90 mg/kg bw/d. Thus, the no observed adverse effect level (NOAEL) for maternal toxicity was set at 50 mg/kg bw/d.

Substance-related, specific adverse effects on foetal morphology as cleft palates were not observed in this study at the tested dose levels up to 90 mg/kg bw/d.

RAC comment:

The findings of 'thoracic centrum fused with arch' in the guinea pig developmental toxicity study should be noted. This effect occurs with a clear dose response (litter incidences 26-52-63-72%) and with the effects in the two highest dose groups being statistically significant.

General RAC comments on malformations

- Cleft palates

The submitter of the AIR suggests that cleft palate increases are an effect of the epoxiconazole treatment in rats at the high dose level. It is also proposed that oestradiol depletion is unlikely to be a primary cause of cleft palates induction in rats. Nevertheless, for the submitter of the AIR the available data suggests that rat-specific placental damage may be a necessary predisposition for cleft palate incidence induced by the epoxiconazole high – dose treatment.

Considering the guinea pig studies' results, the submitter of the AIR explains that:

- The lack of both cleft palates and the lack of any placental damage in guinea pig studies at a dose level of 90 mg/kg bw/d
- Discrepant results from epoxiconazole in vitro exposure during GD 9-11 (dysmorphogenesis, no placental barrier) and in vivo exposure during GD 6-11 (no dysmorphogenesis, placental barrier still intact?)
- The inability to achieve physiological oestradiol levels by the ECP treatment regime employed which may explain why the placental damage at 50 mg/kg bw/d could only be partly reversed

Finally, the submitter of the AIR proposes a classification Repr. 2 (CLP) and Repr. Cat. 3 (Directive 67/548/EEC).

As already stated in the previous RAC opinion of 17 March 2010, cleft palate is a rare malformation.

Exposure to epoxiconazole with co-administration of oestradiol in rats had no impact on the incidence of cleft palates, even though a concurrent decrease was observed in the incidence of post-implantation. Data on kinetics and metabolism

in pregnant guinea pigs were similar to the data obtained from pregnant rats and both species formed the same metabolite 1,2,4-triazole known to be a teratogenic agent (INCHEM, IPSC reviewed April 2007[3]).

However, guinea pigs show general toxicity at lower doses of epoxiconazole compare to rats. Therefore, the absence of cleft palates in guinea pigs could be related to the fact that the dose levels required to achieve the embryonic concentration needed for the induction of cleft palates were above the dose levels tolerated by the adult, pregnant guinea pig.

In summary there is **insufficient information which demonstrates that the occurrence of cleft palates in rats has no relevance to humans; this justifies classification.**

The mode of action for the formation of cleft palates has not been identified. BASF suggests that the degenerative changes in placenta may be implicated in the occurrence of cleft palates in rats. However, it should be noted that cleft palates still occur when such changes are partly compensated by the co-administration of oestradiol (Schneider et al., 2010c). As discussed by Menegola et al (2006), cleft palates are caused by many azoles and could therefore be considered as an azole class effect. For example, in 1998, the Commission Group of Specialized Experts in the fields of the Carcinogenicity, Mutagenicity and Reprotoxicity looked at another triazole, flusilazole, and noted an increase in cleft palate in treated rats. They concluded that although the increase occurred in the presence of maternal toxicity it indicated a clear teratogenic effect that was not necessarily related to maternal toxicity.

- Fusion of thoracic centrum and arch

The submitter of the AIR states that the effects are either not dose related or occur only in the high dose group and that they are caused by delayed ossification which is caused by maternal toxicity stress.

It is not clear how the fusion of thoracic centrum and arch can be caused by a delayed ossification. On the contrary, this may suggest an **advance** in the ossification process of vertebrae when considered with the potential repercussions on the later development of the foetus or pup. Furthermore, there are no signs of maternal toxicity at dose levels where this finding was observed.

RAC also notes that the submitter of the AIR defines this effect on the vertebrae as a *variation*, probably because 6 affected litters were observed also in the control group. However, as the flexibility of the vertebral column may be compromised by the (premature) fusion of centrum and arch, this effect is potentially adverse and can be considered as a malformation. The submitter of the AIR refers to a study by Rocca and Wehner (2009) as supporting the hypothesis that this effect could be a common skeletal abnormality in guinea pigs. However, it should be noted that thoracic centrum fused with arch was not observed in their study. Likewise, this effect is not observed in another study on guinea pigs by the same group (Wehner at al 2009). In RAC's view, this study does not prove absence of teratogenicity in guinea pigs, but rather indicates that guinea pigs are also sensitive to the developmental toxicity of epoxiconazole. This excessive maternal toxicity made any foetal evaluation impossible. Therefore, the potential for a teratogenic effect at high exposures in guinea pigs could not be excluded.

[3] 1,2,4-triazole – INCHEM IPSC - http://www.inchem.org/documents/icsc/icsc/eics0682.htm

In conclusion, the occurrence of cleft palates in the rat foetuses in the presence or absence of overt maternal toxicity, the presence of skeletal findings in guinea pigs (e.g. fusion of thoracic centrum and arch), the absence of a clear mechanism of action supporting the induction of anomalies and the possible relevance of teratogenic action of the azole class to humans is sufficient on its own for classification as **Repr. 1B (CLP) and Repr. Cat. 2 (DSD).**

Comparison with the criteria for classification for reproductive toxicity in the CLP Regulation and DSD

CLP criteria for reproductive toxicants Category 1B

'*Presumed human reproductive toxicant*

*The classification of a substance in category 1B is largely based on data from **animal studies**. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on **development** in the absence of other effects or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effect. However, when there is **mechanistic information** which raises doubt about the **relevance of the effect for humans**, classification in category 2 may be more appropriate*.'

RAC conclusions:

The two main adverse effects: **post implantation losses and late resorptions** and **cleft palates** have been assessed by the Committee and the conclusions are as follows:

- For post implantation losses and late resorptions taking into account the similar effect seen in rats and for another azole (letrozole) in non-human primates and the common mode of action to all test species, as well as the relevance to humans, a classification **Repr. 1B (CLP) and Repr. Cat. 2 (DSD)** is proposed for this effect.
- The presence of cleft palates in the rat foetuses in presence or absence of overt maternal toxicity, the presence of skeletal findings in guinea pigs (e.g. fusion of thoracic centrum and arch), the absence of clear mechanism of action supporting the induction of anomalies and the possible relevance of teratogenicity among some other members of the azole class to humans all support classification as **Repr. 1B (CLP) and Repr. Cat. 2 (DSD).**
- In conclusion, it is proposed to classify **epoxiconazole** as **Repr. 1B (CLP) and Repr. Cat. 2 (DSD).**

ANNEXES

Annex 1	RAC opinion of 17 March 2010 on a dossier proposing harmonised classification and labelling at Community level for epoxiconazole
Annex 2	RAC opinion of 11 March 2011 on certain scientific study plans in relation to epoxiconazole
Annex 3	Request from the Executive Director of ECHA to RAC of 25 April 2012 (I(2012)0222) – 'the mandate'
Annex 4	Summary of the Additional Information Report (AIR) and RAC comments

Annex 5 Comments and response to comments on the AIR (RCOM)

Annex 6 Additional Information Report (AIR)

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